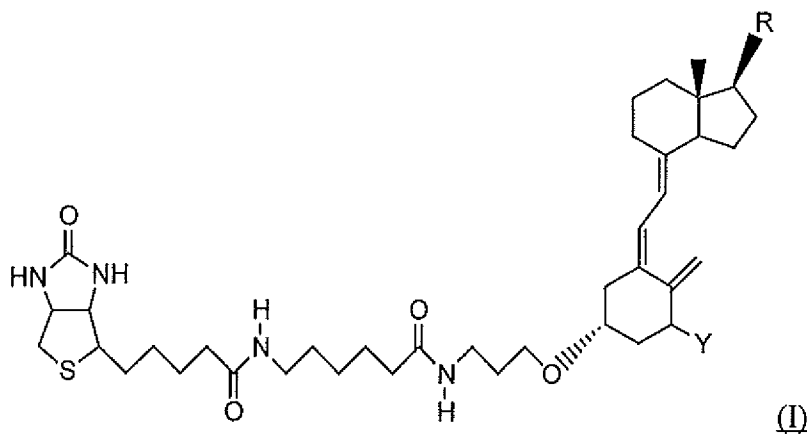
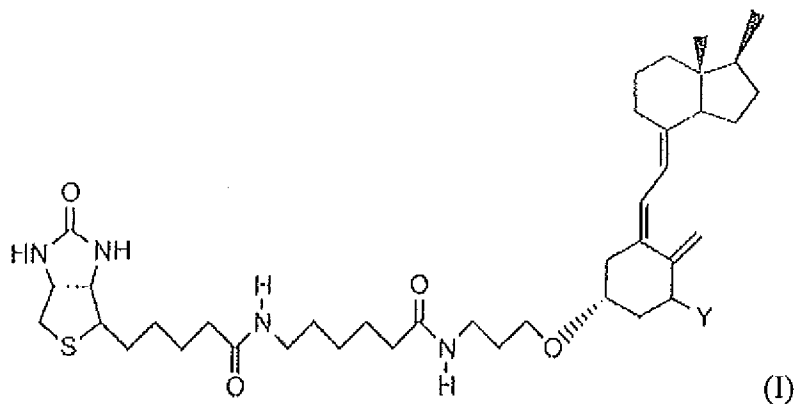


**AMENDMENTS TO THE CLAIMS**

1, - 15. (Canceled)

16. (Currently amended) A method of measuring the amount of  $1\alpha,25$ -dihydroxy vitamin D in human serum using a competitive protein binding assay, comprising:

- i) separating 25-hydroxy vitamin D from  $1\alpha,25$ -dihydroxy vitamin D by binding  $1\alpha,25$ -hydroxy vitamin D in a sample of the human serum to a material that specifically binds  $1\alpha,25$ -hydroxy vitamin D and eluting  $1\alpha,25$ -dihydroxy vitamin D from said material to provide a measurement sample,
- ii) measuring the displacement of a vitamin D derivative of formula (I) from an antibody that specifically binds  $1\alpha,25$ -dihydroxy vitamin D by adding an amount of the measurement sample to a sample of the antibody having the vitamin D derivative of formula (I) bound thereto,



wherein:

R represents a 25-hydroxylated side-group of vitamin D<sub>2</sub> or of vitamin D<sub>3</sub>, and Y represents hydroxy; and

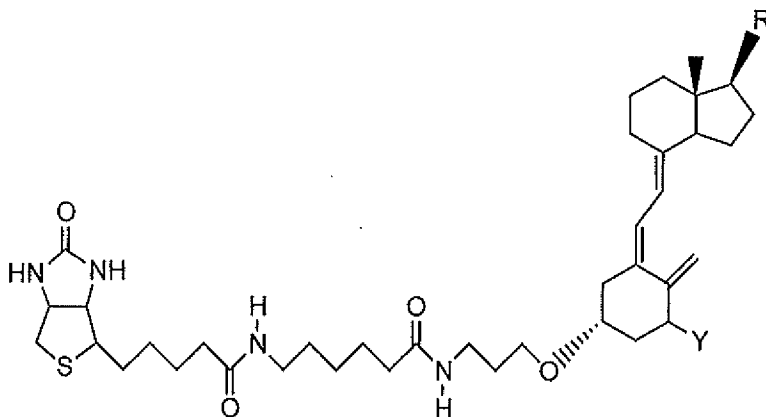
iii) correlating the measurement of displacement of the vitamin D derivative of formula (I) from said antibody by 1 $\alpha$ ,25 dihydroxy vitamin D present in the measurement sample to ~~the a~~ measurement of displacement of the ~~1 $\alpha$ ,25-dihydroxy-vitamin D derivative of formula (I)~~ from the antibody ~~using by~~ a known quantity of the 1 $\alpha$ ,25 dihydroxy vitamin D derivative of formula (I) to determine the amount of 1 $\alpha$ ,25-dihydroxy vitamin D in the sample.

17. - 18. (Canceled)

19. (Previously presented) The method of claim 16, wherein said competitive protein binding assay is selected from the group consisting of an enzyme immunoassay, an enzyme-linked immunosorbent assay, a radioimmunoassay, an immunoradiometric assay, a luminescence assay, a fluorescence immunoassay and an immunofluorometric assay.

20. (Previously presented) The method of claim 16, wherein the method is a sandwich immunoassay, selected from the group consisting of immunoradiometric assay, IEMA/EIA, immunoluminometric assay and immunofluorometric assay.

21. (Previously presented) A kit for determining the concentration of 1 $\alpha$ ,25-dihydroxy vitamin D in a sample of human serum by an immune-based competitive protein binding assay, comprising a standardized quantity of a solid vitamin D derivative of formula (I) or a standardized solution of a vitamin D derivative of formula (I),



wherein R represents a 25-hydroxylated side-group of vitamin D<sub>2</sub> or of vitamin D<sub>3</sub>, and Y represents hydroxy;

a standardized quantity of an antibody that specifically binds 1 $\alpha$ ,25-dihydroxy vitamin D;

and a known quantity of 1 $\alpha$ ,25-dihydroxy vitamin D,

so that the displacement of the vitamin D derivative of formula (I) from said antibody as

effected by the 1 $\alpha$ ,25-dihydroxy vitamin D present in the measurement sample can be

correlated to the displacement of the vitamin D derivative of formula (I) from said

antibody as effected by the addition of a known quantity of the 1 $\alpha$ ,25-dihydroxy vitamin

D to determine the amount of 1 $\alpha$ ,25-dihydroxy vitamin D present in human serum.

22. (Previously presented) The kit of claim 21, further comprising a material that can bind 1 $\alpha$ ,25-dihydroxy vitamin D for separation of 25-hydroxy vitamin D from 1 $\alpha$ ,25-dihydroxy vitamin D.

23. (Previously presented) The kit of claim 21, wherein said competitive protein binding assay is selected from the group consisting of an enzyme immunoassay, an enzyme-linked immunosorbent assay, a radioimmunoassay, an immunoradiometric assay, a luminescence assay, a fluorescence immunoassay and an immunofluorometric assay.

24. (Previously presented) The kit of claim 21, wherein said competitive binding assay is a sandwich immunoassay, selected from the group consisting of immunoradiometric assay, IEMA/EIA, immunoluminometric assay and immunofluorometric assay.

25. (Previously presented) The kit of claim 21 comprising a solid phase selected from the group consisting of a microtitration plate, another solid carrier, a microparticle, a polymeric material, and a cellulose.

26. (Previously presented) The kit of claim 19, in which the solid phase is a microparticle comprising agarose.

27. (Previously presented) The kit of claim 19, in which the solid phase is a magnetic microparticle.

28. (Previously presented) The kit of claim 22, in which the material that can bind  $1\alpha,25$ -dihydroxy vitamin D for separation of 25-hydroxy vitamin D from  $1\alpha,25$ -dihydroxy vitamin D is one suitable for packing into a chromatographic column or one that is provided in a chromatographic column.